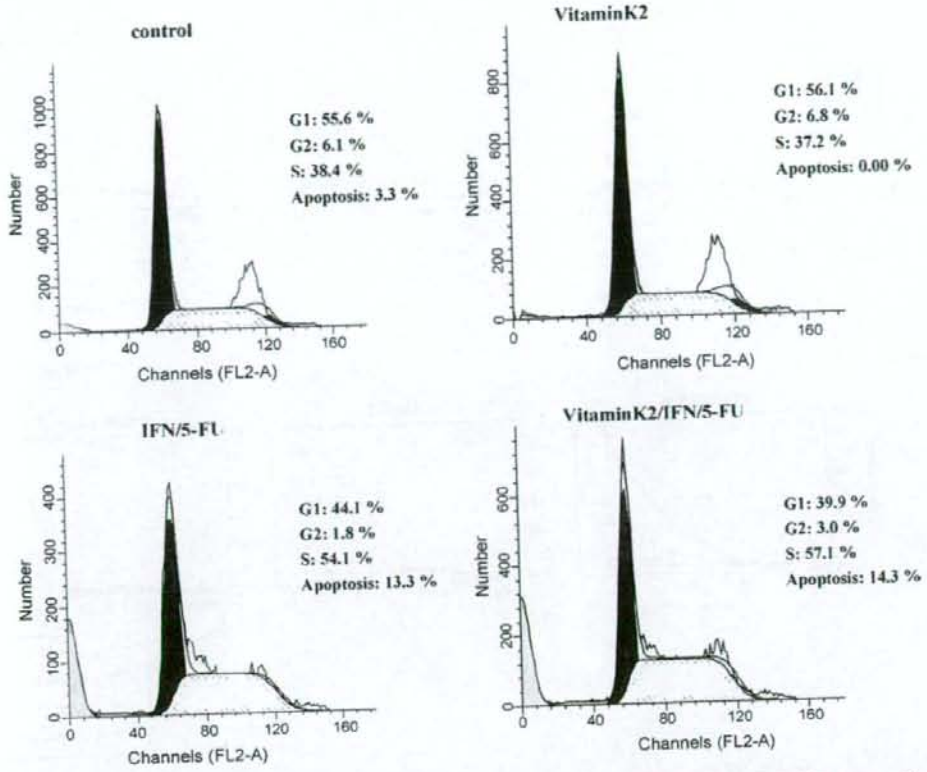

Fig. 4. Flowcytometric analysis of cell cycle of Hep3B on day 5. Doses of IFN and 5-FU was fixed as 500 U/ml and 0.5 μg/ml. There was neither additive nor synergistic effect of Vitamin K2 to IFNα/5-FU in terms of cell cycle arrest.

4. Discussion

Being no satisfactory therapy because of low efficacy and potential complications, best supportive care has been recommended for the patients of HCCs with PVTT. But recently, significant clinical effect of combined IFNα and 5-FU for such far advanced HCC has been reported from several investigators including us [6–10]. According to our data, this combination therapy showed about 50% of response rate against HCC with PVTT and prolonged significantly the survival of responders of this therapy [8,10]. This response rate was exclusively higher than other reported rate of other chemotherapeutic drugs against HCCs [10–12]. But no survival benefit was observed in non-responders that occupied about half of all patients who received same combination therapy unfortunately [8,10]. To rise up the effective rate of this treatment, a new therapeutic modality must be necessary. The focus of the present *in vitro* study was an effort to explore if Vitamin K2 enhances clinical effect of IFNα/5-FU against HCC or not.

Vitamin K2 is a fat-soluble essential vitamin and known as a component of blood coagulation, and recently, apoptotic effect for the tumor cells was also reported including leukemia cells, MDS cells, ovarian cancer cells, pancreas cancer cells and HCC cells *in vitro* [16–22]. Although the mechanisms underlying these apoptotic effects are not revealed, recent studies showed some evidences that involves CDC25A phosphatase, c-Myc phosporylation or protein kinase activity (PKA) [19,23–25]. These factors relates to cell growth and proliferation, especially cell cycle regulation. CDC25A is expressed in early G1 and G2-M phase and it is also reported to have a role in the initiation of mitosis [26]. The c-Myc protein plays a critical role in cell growth and proliferation. PKA induces cell cycle arrest, not only G1 phase, but also G2/M phase, and it is also a regulator of AP-2 and USF-1. They are reported to be related to cell growth or metastasis inhibition [27,28].

In clinical, we reported the relationship of intrahepatic metastasis and high level of PIVKA-II, which is abnormal prothrombin and appears in the situation of Vitamin K deficiency or after administration of Vitamin K antagonists, such as warfarin [29]. Recently, prospective randomized controlled trial showed PIVKA-II is useful not only as tumor marker of HCC but also as predisposing factor for the development of PVTT [30]. Thus it must be a tight correlation between PIVKA-II, Vitamin K and PVTT, and that is the

reason why we selected this agent as the possibility of modification to IFNα/5-FU.

In the present study, we checked direct growth inhibitory effect of Vitamin K2 in both short (48 h) and long term (3–7 days). Growth inhibition was seen in short term means cytotoxic effect including necrosis and apoptosis mainly play a role, as being recognized, growth inhibitory effect in long term suggests cytostatic effect including cell cycle arrest. As shown in the results, Vitamin K2 inhibited growth of all three cell lines after 5 days from stimulation. In the study of additive effect, within 48 h, Vitamin K2 has no enhancement in the effect of cell growth, cell cycle and apoptosis induced by IFNα/5-FU in normal dose. Being considered with the hypothesis which the effect of IFNα/5-FU was much stronger and masked the additive, we performed experiments using IFNα and 5-FU with medium and low dose. The additive effects were observed in IFNα/5-FU/Vitamin K2 arm in these doses only after 5 days; IFNα/5-FU showed anti-tumor effect within 48 h. These results suggested that anti-tumor effect of Vitamin K2 would be cytostatic or growth inhibitory rather than cytotoxic effect. From our data, anti-tumor effect by the addition of Vitamin K2 such as the rapid reduction of tumor volume can not be expected as an effective modality that rises up the response rate of IFNα/5-FU. On the other hand, we have reported the efficacy of IFNα/5-FU in the use of adjuvant setting after curative hepatectomy [9]. In such cases, there might be possibility that Vitamin K2 enhance the anti-tumor effect of IFNα/5-FU to prevent the recurrence.

About the mechanisms of combination of IFNα/5-FU, several pathways were investigated, including cell cycle arrest, apoptosis and immunomodulation [13–15]. As one of these mechanisms, cell cycle arrest effect indicated the delay in the progression of G0–G1 to S phase [13]. In this pathway, up regulation of $p27^{kip1}$ is critical as one of CDK inhibitors was observed. We expected synergistic cell cycle arrest might occur in the anti-tumor effect of Vitamin K2 and IFNα/5-FU. Flowcytometric analysis showed IFNα/5-FU lead cell cycle to S phase and increasing ratio of apoptotic cells that are compatible with our previously report [13]. On the other hand, concerning influence of Vitamin K2, no additive effect was seen. In this point, it has been reported that cell cycle arrest effect is not dramatic that increasing of G1 phase within 10% [19]. Vitamin K2 might work mainly on growth or metastatic inhibition rather than cell cycle arrest.

In conclusion, Vitamin K2 had anti-tumor effect against HCC cell lines, but there was neither additive nor synergistic effect to IFNα/5-FU. According to present data, there may be no possibility to establish new combined therapeutic regimen using Vitamin K2 and IFNα/5-FU against the advanced HCC.

References

[1] Farmer DG, Rosove MH, Shaked A, Busuttil RW. Current treatment modalities for hepatocellular carcinoma. Ann Surg 1994;219:236–47.

[2] Fan J, Wu ZQ, Tang ZY, et al. Multimodality treatment in hepatocellular carcinoma patients with tumor thrombi in portal vein. World J Gastroenterol 2001;7:28–32.

[3] Fujii T, Takayasu K, Muramatsu Y, et al. Hepatocellular carcinoma with portal tumor thrombus: analysis of factors determining prognosis. Jpn J Clin Oncol 1993;23:105–9.

[4] Barnett Jr CC, Curley SA. Ablative techniques for hepatocellular carcinoma. Semin Oncol 2001;28:487–96.

[5] Shen DW, Lu YG, Chin KV, Pastan I, Gottesman MM. Human hepatocellular carcinoma cell lines exhibit multidrug resistance unrelated to MRD1 gene expression. J Cell Sci 1991;98:317–22.

[6] Urabe T, Kaneko S, Matsushita E, Unoura M, Kobayashi K. Clinical pilot study of intrahepatic arterial chemotherapy with methotrexate, 5-fluorouracil, cisplatin and subcutaneous interferon-alpha-2b for patients with locally advanced hepatocellular carcinoma. Oncology 1998;55:39–47.

[7] Sakon M, Nagano H, Dono K, et al. Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. Cancer 2002;94:435–42.

[8] Patt YZ, Hassan MM, Lozano RD, et al. Phase II trial of systemic continuous fluorouracil and subcutaneous recombinant interferon alfa-2b for treatment of hepatocellular carcinoma. J Clin Oncol 2003;21:421–7.

[9] Nagano H, Sakon M, Eguchi H, et al. Hepatic resection followed by IFN-α and 5-FU for advanced hepatocellular carcinoma with tumor thrombus in the major portal branch, Hepatogastroenterology, in press.

[10] Ota H, Nagano H, Sakon M, et al. Treatment of hepatocellular carcinoma with major portal vein thrombosis by combination of subcutaneous interferon-α and intra-arterial 5-fluorouracil. Role of expression of type1 interferon receptor. B J Cancer 2005;93:557–64.

[11] Doci R, Bignami P, Bozzetti F, et al. Intrahepatic chemotherapy for unresectable hepatocellular carcinoma. Cancer 1998;61:1983–7.

[12] Llovet JM, Sala M, Castells L, et al. Randomized controlled trial of interferon treatment for advanced hepatocellular carcinoma. Hepatology 2000;31:54–8.

[13] Eguchi H, Nagano H, Yamamoto H, et al. Augmentation of antitumor activity of 5-fluorouracil by interferon alpha is associated with up-regulation of p27Kip1 in human hepatocellular carcinoma cells. Clin Cancer Res 2000;6:2881–90.

[14] Kondo M, Nagano H, Wada H, et al. Combination of IFNα and 5-fluorouracil induces apoptosis through IFNα/β receptor in human hepatocellular carcinoma cells. Clin Cancer Res 2005;11:1277–86.

[15] Yamamoto T, Nagano H, Sakon M, et al. Partial contribution of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/TRAIL receptor pathway to antitumor effects of interferon-α/5-fluorouracil against hepatocellular carcinoma. Clin Cancer Res 2004;10:7884–95.

[16] Wang Z, Wang M, Finn F, Carr BI. The growth inhibitory effects of vitamin K and their actions on gene expression. Hepatology 1995;22:876–82.

[17] Nishikawa Y, Carr BI, Wang M, et al. Growth inhibition of hepatoma cells induced by Vitamin K and its analogs. J Biol Chem 1995;270:28304–10.

[18] Nishikawa Y, Wang Z, Kerns J, Wilcox CS, Carr BI. Inhibition of hepatoma cell growth in vitro by arylating and non-arylating K vitamin analogs. Significance of protein tyrosine phosphatase inhibition. J Biol Chem 1999;274:34803–10.

[19] Otsuka M, Kato N, Shao RX, et al. Vitamin K2 inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A activation. Hepatology 2004;40:243–51.

[20] Miyazawa K, Yaguchi M, Funato K, et al. Apoptosis/differentiation-inducing effects of Vitamin K2 on HL-60 cells: dichotomous nature of Vitamin K2 in leukemia cells. Leukemia 2001;15:1111–7.

[21] Nishimaki J, Miyazawa K, Yaguchi M, et al. Vitamin K2 induces apoptosis of a novel cell line established from a patient with

myelodysplastic syndrome in blastic transformation. Leukemia 1999; 13:1399–405.
[22] Shibayama-Imazu T, Sakairi S, Watanabe A, Aiuchi T, Nakajo S, Nakaya K. Vitamin K2 selectively induced apoptosis in ovarian TYK-nu and pancreatic MIA PaCa-2 cells out of eight solid tumor cell lines through a mechanism different from geranylgeraniol. J Cancer Res Clin Oncol 2003;129:1–11.
[23] Wang Z, Southwick EC, Wang M, et al. Involvement of CDC25A phosphatase in Hep3B hepatoma cell growth inhibition induced by novel K Vitamin analogs. Cancer Res 2001;61:7211–6.
[24] Ge L, Wang Z, Wang M, Kar S, Carr BI. Involvement of c-Myc in growth inhibition of Hep3B human hepatoma cells by a vitamin K analog. J Hepatol 2004;41:823–9.
[25] Molinari M, Mercurio C, Dominguez J, Goubin F, Dractta GF. Human Cdc25A inactivation in response to S phase inhibition and its role in preventing premature mitosis. EMBO Rep 2000: 71–9.
[26] Molinari M, Mercurio C, Dominguez J, Goubin F, Dractta GF. Human Cdc25A inactivation in response to S phase inhibition and its role in preventing premature mitosis. EMBO Rep 2000:71–9.
[27] Zeng YX, Somasundaram K, el-Deiry WS. AP2 inhibits cancer cell growth and activates p21 WAF1/CIP1 expression. Nat Genet 1997;15:78–82.
[28] Luo X, Sawadogo M. Antiproliferative properties of the USF family of helix-loop-helix transcription factors. Proc Natl Acad Sci USA 1996;93:1308–13.
[29] Sakon M, Monden M, Gotoh M, et al. Relationship between pathologic prognostic factors and abnormal levels of des-gamma-carboxy prothrombin and alpha-fetoprotein in hepatocellular carcinoma. Am J Surg 1992;163:251–6.
[30] Koike Y, Shiratori Y, Sato S, et al. Des-γ-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma. Cancer 2001;91:561–9.

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Hepatoma-Derived Growth Factor Is a Novel Prognostic Factor for Hepatocellular Carcinoma

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Background: Hepatoma-derived growth factor (HDGF) is involved in hepatocarcinogenesis, as well as in liver development and regeneration. This study investigated the correlation of HDGF expression with differentiation and prognosis of hepatocellular carcinoma (HCC).

Methods: HDGF expression in 100 patients with HCC (81 men and 19 women) with ages ranging from 34 to 81 years (median, 61 years) receiving surgical treatment was analyzed by immunohistochemistry. HDGF messenger RNA expression was evaluated in 10 cases by reverse transcription-polymerase chain reaction. The immunostaining pattern in HCCs was categorized as a positive HDGF index (showing positive staining in >90% of tumor cells in both nucleus and cytoplasm) or a negative HDGF index (all others).

Results: Twenty-seven cases (27%) showed a positive and 73 (73%) showed a negative HDGF index. HDGF messenger RNA expression was significantly higher in four cases with a positive HDGF index than in six with a negative index. Cases with well-differentiated histological characteristics showed a higher rate of positive HDGF index than those with a poorly differentiated subtype. Univariate and multivariate analysis revealed significantly poorer disease-free and overall survivals in patients with a positive HDGF index compared with patients with a negative index.

Conclusions: These findings suggest the potential utility of HDGF immunohistochemistry in determining the prognosis of HCC.

Key Words: Hepatoma-derived growth factor—Hepatocellular carcinoma—Prognosis—Recurrence.

Hepatocellular carcinoma (HCC) is one of the most prevalent fatal cancers worldwide, especially in Asia and Africa.[1] Surgical resection offers the chance of a cure, but the prognosis remains poor even in curatively resected cases, mainly because of the high recurrence rate.[2–4] The recurrence rate of HCC after all forms of therapy other than transplantation is 15% to 20% per year and is due to new lesions but not to local recurrence.[2–4] Hence, if

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surgeons could predict or identify patients at high risk for early recurrence, then these patients might be better treated with nonresection therapy. Therefore, the prognostic factors for recurrence and survival are important to help guide clinicians in the management of patients, in the assessment of long-term prognosis, and in the selection of the treatment modality for HCC. Conventionally, the assessment of prognosis in HCC depends on staging by the tumor-node-metastasis system, including tumor morphology and portal vein thrombosis, and the serum level of alfa fetoprotein (AFP).[2–6] Recently, new pathologic and biological factors, including proliferating cell nuclear antigen, Ki-67, and the expression of several genes, including oncogenes and growth factors, have been shown to predict the prognosis of HCC.[7]

Hepatoma-derived growth factor (HDGF) is a heparin-binding protein purified from the conditioned media of HuH-7 hepatoma cells; it proliferates autonomously in a serum-free chemically defined medium.[8,9] HDGF is the first member of the HDGF family of proteins to contain a well-conserved N-terminal amino acid sequence, which is called the *hath* (homologous to amino terminus of HDGF) region.[9,10] HDGF translocates to the nucleus via nuclear localization signals, and its nuclear translocation is essential for the induction of cell growth activity.[11,12] HDGF has mitogenic activity for some HCC cells, in addition to fibroblasts, endothelial cells, vascular smooth muscle cells, and fetal hepatocytes.[8,9,12–17] HDGF antisense oligonucleotides suppress the proliferation of hepatoma cells that express HDGF endogenously.[15] HDGF was more abundantly expressed in HCC than in the nontumorous adjacent liver tissues in human and murine samples.[18] HDGF is a unique nuclear/growth factor that may play an important role in the development and progression of HCC. In this study, the expression level of HDGF in HCC was examined by reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical analysis, and its correlation with recurrence and survival in patients with HCC was evaluated.

PATIENTS AND METHODS

Patients and Tissue Samples

One hundred patients who received curative resection for primary HCC at the Gastroenterological Surgery Division, Osaka University Hospital, from October 1987 to January 2001 were analyzed for this study. There were 81 men and 19 women with ages ranging from 34 to 81 years (median, 61 years). Sixteen patients were positive for hepatitis B virus surface antigen, and 66 were positive for hepatitis C virus antibody. Preoperative diagnostic imaging examinations, including ultrasonography, computed tomographic scan, and angiography, were performed in all patients. Liver function was assessed according to the Child-Pugh classification. Types of surgery used were limited resection in 46 patients, subsegmentectomy in 24, segmentectomy in 17, lobectomy in 12, and extended lobectomy in 1.

Surgically resected specimens were fixed in 10% formalin, macroscopically examined, and sliced at 5-mm intervals. The section containing the largest volume of HCC was processed for paraffin embedding. Four to 43 blocks per case were obtained. Histological sections cut at 4-μm thicknesses were stained with hematoxylin and eosin and reviewed by two of the authors (K.Y. and Y.T.) to determine the following categories: differentiation of tumor cells based on the criteria proposed by Edmondson and Steiner[19] (I, well differentiated; II, moderately differentiated; III, poorly differentiated; IV, undifferentiated), pattern of growth (expansive or infiltrative), formation of a fibrous capsule around the tumor, portal vein invasion, tumor multiplicity, and positivity for the surgical margin. The surgical margin was identified as positive when tumor cells were present at the edge. The degree of inflammation and fibrosis in noncancerous hepatic tissues was shown as the histological activity index score.[20] The representative one slide per case was used for HDGF immunohistochemistry.

After resection, all patients were followed up by monitoring serum AFP, ultrasonography, and contrast-enhanced computed tomographic scan every 1 and 3 months; for suspicious cases, angiography was performed to verify the recurrence. The follow-up periods for survivors ranged from 2 to 128 months (median, 43 months) after surgery.

Anti-Human HDGF Antibody and Western Blotting

Rabbit polyclonal antibody was raised against C-terminal amino acids (amino acids 231–240) of the human HDGF sequence. The specificity and sensitivity of the antibody have been described previously.[10,13] Protein samples were extracted from human cell lines, HepG2, PLC/PRF/5, HuH7, and HT29 by using CelLytic-M Mammalian Cell Lysis/

Extraction Reagent (Sigma, St. Louis, MO). Ten micrograms of cell lysates, along with recombinant HDGF, was electrophoresed in sodium dodecyl sulfate polyacrylamide gel and transblotted onto polyvinylidene difluoride transfer membranes (Millipore, Bedford, MA). The blotted membranes were reacted with an affinity-purified polyclonal anti-C-terminus of HDGF antibody generated by rabbit at a dilution of 1/10,000 and then visualized with an electrochemiluminescence detection system (Amersham Pharmacia Biotech, Buckinghamshire, UK).

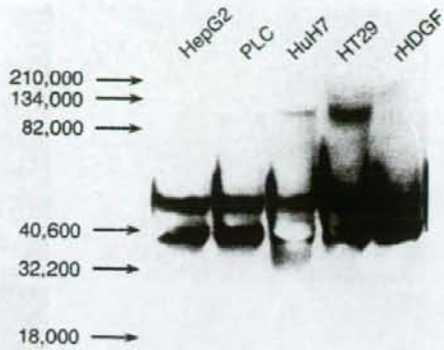

FIG. 1. Western blotting of hepatoma-derived growth factor (HDGF). All four cell lines and recombinant HDGF showed double bands of 43 and 39 kDa.

Immunohistochemical Analysis

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections by using the avidin-biotin complex method. Antigen retrieval was performed with microwave treatment (5 minutes, three times) in 10 mM of citrate buffer (pH 6.0). Anti-HDGF antibody was used as the primary antibody at a dilution of 1/5000. Sections were lightly counterstained with methyl green. Positive staining in the bile ducts in the noncancerous lesions was used as the internal positive control. Stained sections were evaluated in a blinded manner without prior knowledge of the clinicopathologic parameters. The counting of immunohistochemically positive cells was performed by hand under a microscope. For each case, all the HCC area in the slides was carefully examined, and the HDGF-positive rate was determined.

The HDGF expression pattern was independently evaluated for the nucleus and cytoplasm; cells showing a staining intensity similar to or stronger than that in bile ducts in the nucleus or cytoplasm were regarded as nucleus positive or cytoplasm positive, respectively. Samples with >90% of tumor cells that expressed positive immunoreactivity both for nucleus and cytoplasm were regarded as HDGF index positive, and others were regarded as HDGF index negative.

Quantitative RT-PCR Analysis of HDGF

Total RNA was extracted from fresh-frozen samples in 18 cases of HCC with TRIzol reagent (Invitrogen, Carlsbad, CA). Ten micrograms of deoxyribonuclease I–treated total RNA was used for RT with Superscript II (Invitrogen). An aliquot representing 100 ng of input RNA was amplified by quantitative real-time PCR by using a TaqMan PCR Reagent Kit (Applied Biosystems, Foster City, CA) with the ABI PRISM 7700 Sequence Detection System (Applied Biosystems) as follows: 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The following were used for amplification of β-actin: forward primer, 5′-TCACCCACACTGTGCCCATC TACGA-3′; reverse primer, 5′-CAGCGGAACCGCT CATTCGCCAATGG-3′; and probe, 5′—6-carboxy-fluorescein (FAM)-ATGCCC—6-carboxytetramethylrhodamine (TAMRA)-CCCCCATGCCATCCTG CGTp-3′. The forward primer 5′-AAGTTTGGCAA GCCCAACA-3′, reverse primer 5′-GGCTCTTCCA CACAGCTCTTT-3′, and probe 5′-FAM-AACCC TACTGTCAAGGCTTCCGGCT-TAMRA-3′ were used for HDGF. RNA extracted from an HCC sample in one case was used as a standard. After RT, standard complementary DNA (cDNA) was serially diluted to obtain five standard solutions for a use in PCR reaction to generate the reference curve. The relative amount of cDNA in each sample was measured by interpolation in the standard curve, and then the relative ratio of HDGF/β-actin expression was calculated for each HCC sample.

Statistics

Statistical analysis was performed by using JMP (SAS Institute Inc., Cary, NC). The correlation between the expression level of HDGF at quantitative RT-PCR and immunohistochemistry was evaluated by one-way analysis of variance. Correlations between the HDGF expression level by immunohistochemistry and the clinicopathologic parameters were evaluated by χ^2 test and Fisher's exact probability test. The overall and disease-free

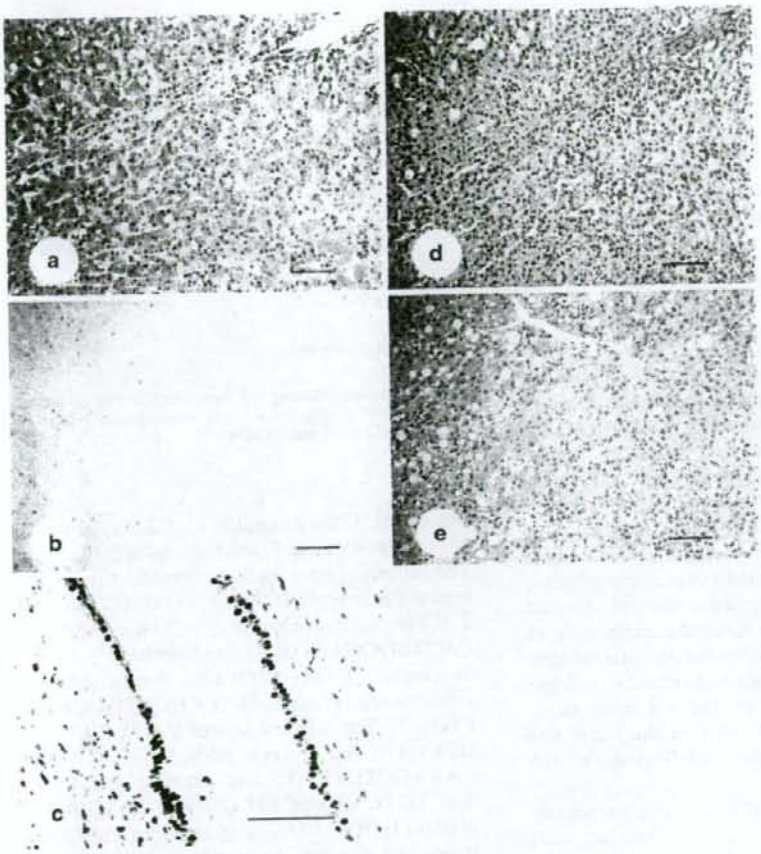

FIG. 2. Hepatoma-derived growth factor (HDGF) immunohistochemistry. (a and d) Hepatocellular carcinoma (HCC) with a positive HDGF index. More than 90% of the tumor cells showed positive nuclear/cytoplasmic staining. (b and e) HCC with a negative HDGF index. Most tumor cells did not express HDGF. (c) Internal control of HDGF. The bile duct is positively stained in the nucleus and cytoplasm (bar = 100 mm).

survival rates were calculated by using Kaplan-Meier methods,[21] and differences in survival curves were analyzed by the log-rank test. Independent prognostic factors were analyzed by the Cox proportional hazards regression model in a stepwise manner.[22] $P < .05$ was considered statistically significant.

RESULTS

Western Blotting

Western blotting by using HDGF antibody showed double bands sized 43 and 39 kDa in all lanes, including that of recombinant HDGF. This suggests the specificity and sensitivity of the antibody used in this analysis (Fig. 1).

HDGF Expression Pattern in HCC

Forty-two cases (42%) showed strong staining in the nucleus of >90% of tumor cells, which were thus regarded as nucleus-positive; 46 cases with strong cytoplasmic staining in >90% of tumor cells were cytoplasm positive. Among them, 27 cases (27%) were both nucleus and cytoplasm positive and were thus regarded as having a positive HDGF index. The remaining 73 cases (73%) were regarded as having a negative HDGF index (Fig. 2).

RNA was extracted from 18 cases with HCC, and they were tested for RNA preservation by β-actin RT-PCR. Ten of the 18 cases showed an increase of the reaction curve before 25 PCR cycles, and then they were regarded as adequately preserved cases. The remaining eight cases showed an increase of the reaction curve after 25 cycles or showed no increase.

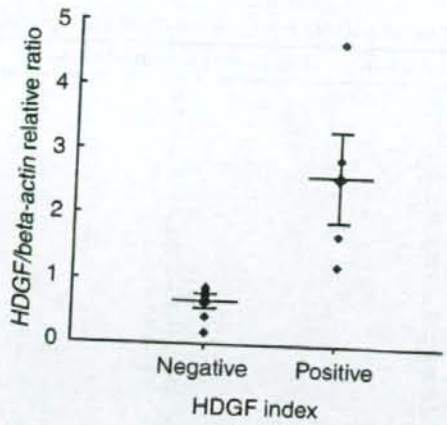

FIG. 3. Hepatoma-derived growth factor (HDGF)/β-actin messenger RNA expression ratio in hepatocellular carcinoma–positive and –negative HDGF indexes by immunohistochemistry. All cases with a positive HDGF index showed higher ratios than those with a negative HDGF index ($P < .05$). Bars are mean ± SD.

They were regarded as having poor RNA preservation and were excluded from this study. The 10 cases consist of 4 positive and 6 negative HDGF indices by immunohistochemistry. The relative ratio of HDGF/β-actin expression in cases with a negative and positive HDGF index was .64 ± .11 and 2.67 ± .71 (mean ± SD), respectively ($P < .05$; Fig. 3).

Relationship Between Clinicopathologic Features and HDGF Expression in HCC

The relationship between HDGF expression and the clinicopathologic features was analyzed (Table 1). The HDGF staining pattern was significantly correlated with the differentiation of HCC. Among 87 HCCs with Edmondson's differentiation grade I, II, and III, 26 (29.9%) had a positive HDGF index, as did 1 (7.7%) of 13 cases with Edmondson's grade IV ($P = .0478$). There was no significant relationship between HDGF expression and other clinicopathologic features.

Univariate and Multivariate Analysis for Prognostic Factors for HCC

The 5-year disease-free and overall survival rates of the 100 patients with HCC were 24.0% and 54.0%, respectively. Tumor recurrence was found in 73 patients. The prognostic significance of HDGF expression in HCC was analyzed for disease-free and overall survival. Patients with HDGF-negative HCC showed significantly better 5-year survival rates than those with HDGF-positive HCC (disease-free survival rate, 34.2% vs. 6.56%, $P = .0149$; overall survival rate, 60.4% vs. 48.9%, $P = .0477$; Fig. 4). Furthermore, when patients with differentiated HCC except for Edmondson's grade IV were solely analyzed, the prognostic significance of the HDGF index was strong (disease-free survival rate, $P = .0087$; overall survival rate, $P = .0474$; Fig. 5).

Tumor multiplicity, portal vein invasion, and serum AFP level were significant factors for disease-free and overall survival (Table 2). The pattern of tumor growth is a prognosticator for disease-free survival but not for overall survival, and the histological stage of fibrosis in the adjacent noncancerous liver tissues (histological activity index: 0 or 1 vs. 3 or 4) significantly affected overall survival but not disease-free survival.

Multivariate analysis was performed with factors proven to be significant in the univariate analysis. HDGF expression, tumor multiplicity, and serum AFP level were independent prognostic factors for disease-free and overall survival (Table 3). The pattern of tumor growth and portal vein invasion were independent prognosticators for disease-free survival, but not for overall survival.

DISCUSSION

Previously, we have demonstrated that exogenous HDGF stimulated the proliferation of HCC cell lines, and its overexpression enhanced the proliferation of HCC cells. Furthermore, the suppression of endogenous HDGF production by antisense oligonucleotides or cDNA inhibited the proliferation of cells expressing HDGF.[9,11,13,15] These findings suggest the importance of HDGF in HCC development; therefore, this analysis was designed to investigate the correlation of HDGF expression with other clinicopathologic factors and its potential utility as a prognostic factor for HCC.

Western blotting by using HDGF antibody showed positive bands at the same size of recombinant HDGF in all cell lines examined. HDGF expression in 10 patients was examined by combined quantitative RT-PCR and immunohistochemical analyses, and these showed a correlation of HDGF expression between messenger RNA (RT-PCR) and protein (immunohistochemistry) levels. However, the sample size used for RT-PCR in this analysis was small, and this should be reassessed in a large number of patients.

The characteristics of the patients in this study with HCC, such as sex, age, and 5-year survival rates, were

TABLE 1. *The relationship between hepatoma-derived growth factor (HDGF) expression and clinicopathologic factors*

Factor	Category	Total no. of patients	Patients with positive HDGF index	*P* value
Age (y)	≤ 60	47	12	NS
	> 60	53	15	
Sex	Male	81	22	NS
	Female	19	5	
HBs-Ag	Positive	17	5	NS
	Negative	83	22	
HCV-Ab	Positive	66	19	NS
	Negative	34	8	
Child-Pugh classification	A	81	24	NS
	B	19	3	
	C	0	0	
AFP (ng/mL)	≤ 200	69	17	NS
	> 200	31	10	
TAE	Performed	46	11	NS
	Not performed	54	16	
Type of operation	Limited resection	46	12	NS
	Subsegmentectomy	24	7	
	Segmentectomy	17	7	
	Lobectomy	12	0	
	Extended lobectomy	1	1	
Tumor size (cm)	≤ 2	16	2	NS
	> 2	84	25	
Tumour multiplicity	Solitary	58	15	NS
	Multiple	42	12	
Differentiation	I	12	6	.0478
	II	39	10	
	III	36	10	
	IV	13	1	
Pattern of tumor growth	Expansive	74	20	NS
	Infiltrative	26	7	
Formation of fibrous capsule	Present	81	19	NS
	Absent	19	8	
Portal vein invasion	Present	55	14	NS
	Absent	45	13	
Stage (pTNM)	I	16	3	NS
	II	58	18	
	III	26	6	
	IV	0	0	
Cirrhosis	Present	35	11	NS
	Absent	65	16	
Inflammatory status (HAI)	Absent (0)	6	1	NS
	Mild (1–3)	28	5	
	Moderate (4–7)	59	21	
	Severe (≥8)	7	0	
Degree of fibrosis (HAI)	Absent (0)	9	1	NS
	Mild (1)	22	7	
	Severe (3)	34	7	
	Cirrhosis (4)	35	12	

NS, not significant; HBs-Ag, hepatitis B virus surface antigen; HCV-Ab, hepatitis C virus antibody; AFP, alfa fetoprotein; TAE, transcatheter arterial embolization; pTNM, pathologic tumor-node-metastasis; HAI, histological activity index.

similar to those in previous studies from Japan[23] and Western countries.[2–4] The present univariate and multivariate analyses confirmed the prognostic significance of tumor multiplicity, serum AFP level, pattern of tumor growth, and portal vein invasion, as reported previously.[5–7] These findings suggest that the results obtained from this study are generally applicable to HCC.

The prognostic significance of HDGF staining was evaluated for nucleus, cytoplasm, and combined nucleus and cytoplasm. The HDGF index value was mostly significant when the cases were divided into cases with staining both for nucleus and cytoplasm in >90% of tumor cells as a positive index and and others as a negative index, and then we used this cutoff level in this study. Other cutoff levels used in this study were 75% and 50%. Furthermore, the prognostic value was mostly significant when cases that were HDGF positive for both the nucleus and cytoplasm and others were compared. This categorization was chosen in this study. A significant correlation was observed between the HDGF index and

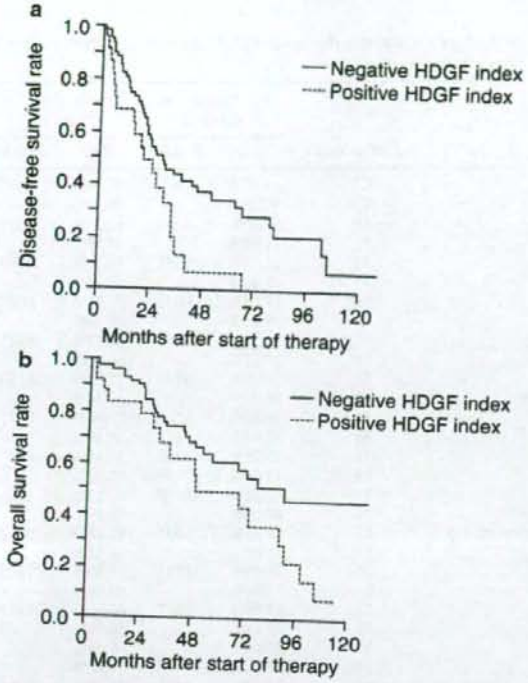

FIG. 4. Disease-free (a) and overall (b) survival curves of patients with hepatocellular carcinoma–positive and –negative hepatoma-derived growth factor (HDGF) indexes. A significant difference was observed between groups (a, $P = .0149$; b, $P = .0477$).

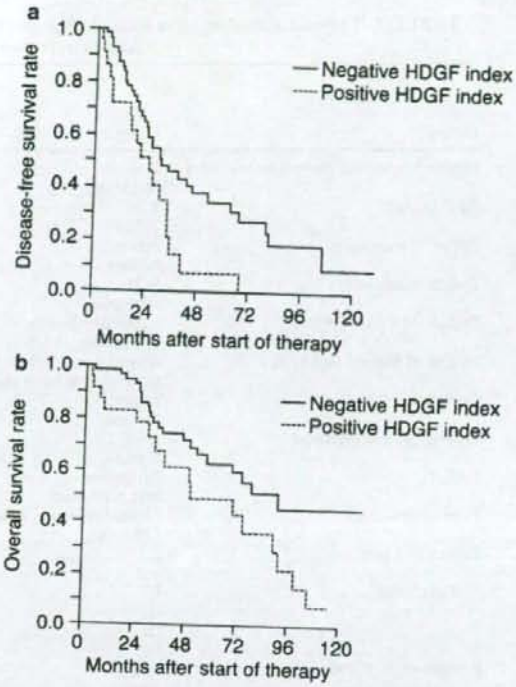

FIG. 5. Disease-free (a) and overall (b) survival curves of patients with differentiated hepatocellular carcinoma (Edmondson's grade I, II, and III)–positive and –negative hepatoma-derived growth factor (HDGF) indexes. A significant difference was observed between groups (a, $P = .0087$; b, $P = .0474$).

tumor differentiation. Only one HCC with a poorly differentiated subtype (Edmondson grade IV) had a positive HDGF index, whereas 26 of 87 with differentiated group (Edmondson grade I–III) had a positive HDGF index.

Patients with Edmondson's grade IV differentiation showed the poorest prognosis and the highest rate of portal vein invasion compared with the others; however, the difference was not significant. In addition, the prognostic significance of the HDGF index was stronger when patients with Edmondson's grade I and II differentiation were solely analyzed. HDGF might be a prognostic marker of HCC, especially for cases with a well-differentiated subtype. Additional studies with more patients are necessary to clarify the inverse effect of HDGF expression and tumor grade on prognosis. It has been demonstrated that HCC initially develops in the form of a well-differentiated subtype in cirrhosis or hepatitis, from which a histologically less-differentiated subgroup might occur and gradually replace the well-differentiated tumor.[24–26] Similarly, a significantly decreased expression of transforming growth factor α, epidermal growth factor receptor, and cyclooxygenase 2 in poorly differentiated HCC was shown when compared with well-differentiated HCC.[27,28]

The univariate and multivariate analyses demonstrated that the HDGF index was an independent prognosticator for HCC patients. The main cause for the poor prognosis of HCC is tumor recurrence in the liver.[2–4] HDGF works for HCC proliferation, and, in addition, stimulation of the endothelial cell proliferation by HDGF was observed in renal and cardiovascular development and tumor formation in vivo, thus suggesting its involvement in angiogenesis.[14,16,17] These HDGF functions are convenient for HCC cells to invade into the microvascular system and survive to form recurrent foci. These findings suggest that increased HDGF expression is a sign of poor prognosis in HCC. HDGF index, tumor multiplicity, and

TABLE 2. *Univariate analysis of individual clinicopathologic factors for disease-free and overall survival in patients after surgical resection of hepatocellular carcinoma*

Factor	Category	No. of patients	5-y disease-free survival		5-y overall survival	
			Rate	*P* value	Rate	*P* value
Hepatoma-derived growth factor index	Positive	27	6.56%	.0149	48.94%	.0477
	Negative	73	34.20%		60.42%	
AFP (ng/mL)	≤ 200	69	34.35%	.0391	62.53%	.0211
	>200	31	11.83%		45.90%	
Portal vein invasion	Present	55	11.75%	.0351	49.16%	.0465
	Absent	45	49.01%		67.43%	
Tumor multiplicity	Solitary	58	39.60%	.0043	71.83%	.0007
	Multiple	42	8.35%		37.14%	
Pattern of tumor growth	Expansive growth	74	31.51%	.035	63.37%	.4985
	Infiltrative growth	26	27.27%		41.10%	
Degree of fibrosis (HAI)	Absent to mild (0 or 1)	31	38.29%	.3869	79.65%	.0194
	Severe to cirrhosis (3 or 4)	69	26.79%		50.18%	
Cirrhosis	Absent	65	31.88%	.67	72.90%	.0181
	Present	35	27.65%		39.40%	
Child-Pugh classification	A	81	25.26%	.3498	57.19%	.665
	B and C	19	43.64%		65.82%	
TAE	Performed	46	32.55%	.4206	61.27%	.1698
	Not performed	54	24.62%		58.84%	
Type of operation	Limited resection to segmentectomy	87	29.75%	.1401	61.48%	.0762
	Lobectomy	13	13.64%		36.35%	
Tumor size (cm)	≤ 2	16	29.46%	.8515	55.00%	.7643
	>2	84	32.84%		60.84%	
Differentiation	I	12	23.09%	.7547	40.00%	.1313
	II	39	33.68%		66.98%	
	III	36	27.34%		60.69%	
	IV	13	30.77%		54.40%	
Formation of fibrous capsule	Present	81	33.80%	.4458	58.23%	.8295
	Absent	19	0.00%		54.40%	
Stage (pTNM)	I	16	34.72%	.061	63.64%	.0873
	II	58	42.08%		68.76%	
	III	26	0.00%		43.29%	
	IV	0	0.00%		0.00%	
Inflammatory status (HAI)	Absent to mild (0–3)	34	27.09%	.2181	68.57%	.1905
	Moderate to severe (≥ 4)	66	34.00%		54.61%	

AFP, alfa fetoprotein; HAI, histological activity index; TAE, transcatheter arterial embolization; pTNM, pathologic tumor-node-metastasis.

TABLE 3. *Multivariate analysis of individual clinicopathologic factors for disease-free and overall survival in patients after surgical resection of hepatocellular carcinoma*

Factor	Category	Relative risk	95% Confidence interval	χ^2 value	*P* value
Disease-free survival					
HDGF index	1: Positive 0: Negative	3.127	1.698–5.760	13.388	.0003
Pattern of tumor growth	1: Infiltrative growth 0: Expansive growth	2.518	1.389–4.565	9.253	.0024
Portal vein invasion	1: Present 0: Absent	2.289	1.242–4.219	7.042	.008
Tumor multiplicity	1: Multiple 0: Solitary	2.189	1.208–3.968	6.666	.0098
AFP (ng/mL)	1: >200 0: ≤ 200	2.012	1.12–3.601	5.546	.0185
Overall survival					
HDGF index	1: Positive 0: Negative	2.331	1.12–4.491	6.401	.0114
Tumor multiplicity	1: Multiple 0: Solitary	3.211	1.675–6.157	12.34	.0004
AFP (ng/mL)	1: >200 0: ≤ 200	2.61	1.33–5.117	7.8	.0052

HDGF, hepatoma-derived growth factor; AFP, alfa fetoprotein.

serum AFP level were independent prognosticators for both disease-free and overall survival. The combination of these factors might be a useful tool for predicting prognosis and choosing appropriate therapeutic modalities, including liver transplantation, for patients with HCC.[29]

In summary, this study demonstrates an increased rate of a positive HDGF index in well-differentiated HCC compared with poorly or undifferentiated subtypes and its potential prognostic utility for disease-free and overall survival with HCC.

REFERENCES

1. Parkin DM, Muir CS. *Cancer Incidence in Five Continents: Comparability and Quality of Data. Lyon*: International Agency for Research on Cancer Scientific Publications, 1992; 45–173.
2. Fong Y, Sun RL, Jarnagin W, Blumgart LH. An analysis of 412 cases of hepatocellular carcinoma at a Western center. *Ann Surg* 1999; 229:790–9.
3. Lai EC, Fan ST, Lo CM, Chu KM, Liu CL, Wong J. Hepatic resection for hepatocellular carcinoma. An audit of 343 patients. *Ann Surg* 1995; 221:291–8.
4. Lau H, Fan ST, Ng IO, Wong J. Long term prognosis after hepatectomy for hepatocellular carcinoma: a survival analysis of 204 consecutive patients. *Cancer* 1998; 83:2302–11.
5. Poon RT, Fan ST, Lo CM, et al. Improving survival results after resection of hepatocellular carcinoma: a prospective study of 377 patients over 10 years. *Ann Surg* 2001; 234:63–70.
6. Vauthey JN, Lauwers GY, Esnaola NF, et al. Simplified staging for hepatocellular carcinoma. *J Clin Oncol* 2002; 20:1527–36.
7. Ng IO. Prognostic significance of pathological and biological factors in hepatocellular carcinoma. *J Gastroenterol Hepatol* 1998; 13:666–70.
8. Nakamura H, Kambe H, Egawa T, et al. Partial purification and characterization of human hepatoma-derived growth factor. *Clin Chim Acta* 1989; 183:273–84.
9. Nakamura H, Izumoto Y, Kambe H, et al. Molecular cloning of complementary DNA for a novel human hepatoma-derived growth factor. Its homology with high mobility group-1 protein. *J Biol Chem* 1994; 269:25143–9.
10. Izumoto Y, Kuroda T, Harada H, Kishimoto T, Nakamura H. Hepatoma-derived growth factor belongs to a gene family in mice showing significant homology in the amino terminus. *Biochem Biophys Res Commun* 1997; 238:26–32.
11. Kishima Y, Yamamoto H, Izumoto Y, et al. Hepatoma-derived growth factor stimulates cell growth after translocation to the nucleus by nuclear localization signals. *J Biol Chem* 2002; 277:10315–22.
12. Everett AD, Stoops T, McNamara CA. Nuclear targeting is required for hepatoma-derived growth factor-stimulated mitogenesis in vascular smooth muscle cells. *J Biol Chem* 2001; 276:37564–8.
13. Enomoto H, Yoshida K, Kishima Y, et al. Hepatoma-derived growth factor is highly expressed in developing liver and promotes fetal hepatocyte proliferation. *Hepatology* 2002; 36:1519–27.
14. Everett AD, Lobe DR, Matsumura ME, Nakamura H, McNamara CA. Hepatoma-derived growth factor stimulates smooth muscle cell growth and is expressed in vascular development. *J Clin Invest* 2000; 105:567–75.
15. Kishima Y, Yoshida K, Enomoto H, et al. Antisense oligonucleotides of hepatoma-derived growth factor (HDGF) suppress the proliferation of hepatoma cells. *Hepatogastroenterology* 2002; 49:1639–44.
16. Oliver JA, Al-Awqati Q. An endothelial growth factor involved in rat renal development. *J Clin Invest* 1998; 102: 1208–19.
17. Okuda Y, Nakamura H, Yoshida K, et al. Hepatoma-derived growth factor induces tumorigenesis in vivo through both direct angiogenic activity and induction of vascular endothelial growth factor. *Cancer Sci* 2003; 94:1034–41.
18. Yoshida K, Nakamura H, Okuda Y, et al. Expression of hepatoma-derived growth factor in hepatocarcinogenesis. *J Gastroenterol Hepatol* 2003; 18:1293–301.
19. Edmondson H, Steiner P. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer* 1954; 7:462–503.
20. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; 1:431–5.
21. Kaplan E, Meier P. Non-parametric estimation for incomplete observations. *J Am Stat Assoc* 1958; 53:457–81.
22. Cox DR. Regression models and life tables. *J R Stat Soc* 1972; 34:187–220.
23. The Liver Study Group of Japan. Predictive factors for long term prognosis after partial hepatectomy for patients with hepatocellular carcinoma in Japan. *Cancer* 1994; 74: 2772–80.
24. Fusenig NE, Breitkreutz D, Boukamp P, Tomakidi P, Stark HJ. Differentiation and tumor progression. *Recent Results Cancer Res* 1995; 139:1–19.
25. Sakamoto M, Hirohashi S, Shimosato Y. Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. *Hum Pathol* 1991; 22:172–8.
26. Sugihara S, Nakashima O, Kojiro M, Majima Y, Tanaka M, Tanikawa K. The morphologic transition in hepatocellular carcinoma. A comparison of the individual histologic features disclosed by ultrasound-guided fine-needle biopsy with those of autopsy. *Cancer* 1992; 70:1488–92.
27. Koga H, Sakisaka S, Ohishi M, et al. Expression of cyclooxygenase-2 in human hepatocellular carcinoma: relevance to tumor dedifferentiation. *Hepatology* 1999; 29:688–96.
28. Morimitsu Y, Hsia CC, Kojiro M, Tabor E. Nodules of less-differentiated tumor within or adjacent to hepatocellular carcinoma: relative expression of transforming growth factor-alpha and its receptor in the different areas of tumor. *Hum Pathol* 1995; 26:1126–32.
29. Nart D, Arikan C, Akyildiz M, et al. Hepatocellular carcinoma in liver transplant era: a clinicopathologic analysis. *Transplant Proc* 2003; 35:2986–90.

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Case report

A case of hepatocellular carcinoma with multiple lung, spleen, and remnant liver metastasis successfully treated by combination chemotherapy with the novel oral DPD-inhibiting chemotherapeutic drug S-1 and interferon-α

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A 54-year-old man was admitted Osaka University Hospital for hepatocellular carcinoma (HCC) with portal vein thrombus and multiple intrahepatic metastases that extended to the bilateral lobes of the liver. He underwent multimodal therapy, including extended left lobectomy followed by intra-arterial 5-fluorourcil (5-FU) infusion chemotherapy combined with subcutaneous interferon-α (IFN-α) to treat the lesions in the residual liver. Seven months after the initial resection, recurrent tumors in the spleen, lung, and residual liver were detected by follow-up examination. We started a new regimen of per oral administration of S-1 and subcutaneous IFN-α injection, because the combined therapy with intra-arterial 5-FU infusion was not considered effective for distant metastases. After two cycles of S-1 and IFN-α, the metastatic tumor in the spleen and the recurrence in the residual liver had disappeared, and the diagnosis was complete remission with no adverse effect; the pulmonary metastasis showed a partial response, and was finally resected. This patient is still alive with no recurrence 32 months after initial hepatic resection. This outcome suggests that combination therapy with S-1 and IFN-α may be a promising treatment modality against advanced HCC with distant metastasis.

Key words: hepatocellular carcinoma, distant metastasis, interferon, S-1

Introduction

Hepatocellular carcinoma (HCC) is one of the most common solid tumors.[1] Its prognosis, especially in advanced cases with extrahepatic metastasis, is extremely poor.[2] Although chemotherapy is often used for such advanced cases, there are few reports about the efficacy of chemotherapy in terms of HCC tumor regression and prolonged survival.[3,4] No standard therapy for far advanced HCC has emerged, but recently we and others have observed antitumor effects of combination therapy with interferon-α (IFN-α) and 5-fluorouracil (5-FU), clinically and in vitro.[5–12] This combination therapy has demonstrated a response rate of about 50% and has prolonged the survival of patients with far advanced HCC and portal vein tumor thrombus (PVTT). However, the patients with extrahepatic metastasis of HCC have not been included in the indication for this combination therapy, because intrahepatic infusion chemotherapy is used to increase the concentration of anticancer agent in the liver. Thus, a new regimen against systemic metastasis of HCC is strongly needed.

In this article, we report a case of HCC with multiple lung and spleen metastasis, successfully treated by a combination therapy of the novel dihydropyrimidine dehydrogenase (DPD) inhibitory per oral anticancer agent S-1 and IFN-α.

Case

A 54-year-old man was admitted to Osaka University Hospital for HCC in May 2003. Abdominal computed tomography (CT) showed a main tumor infiltrating in the left and caudate lobes, a tumor thrombus in the main branch of the portal vein, and intrahepatic metastasis in segment 8 (Fig. 1a, b). Angiography revealed multiple lesions in the right lobe of the liver, and portography showed PVTT extending from the left lobe and spreading into the right branch via the main trunk of the portal vein (Fig. 1c, d). Tumor marker levels were high (Table 1). We started a multimodal treatment strategy for HCC

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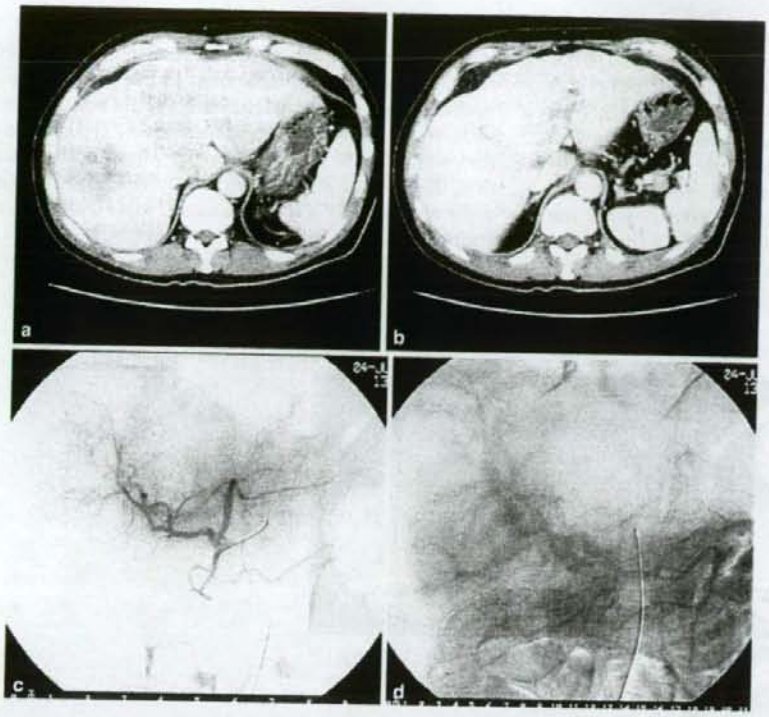

Fig. 1. **a, b** Abdominal computed tomography. **c, d** Hepatic arteriography and portography. **a** Abdominal computed tomography showed the main tumor infiltrating in the left and caudate lobes and an intrahepatic metastasis in the right lobe. **b** The left branch of the portal vein was filled with tumor thrombus. **c** Angiography revealed multiple lesions in the right lobe of the liver. **d** Portography showed a portal vein tumor thrombus, and that growth had begun in the left lobe and spread into the right branch via the main trunk of the portal vein

Table 1. Laboratory results at the time of hospital admission

WBC	8680	/μl	AST	66	IU/l
RBC	461×10^4	/μl	ALT	65	IU/l
Hb	13.6	g/dl	γGT	99	IU/l
Ht	43.8	%	ALP	177	IU/l
Plt	17.6×10^4	/μl	LDH	448	IU/l
PT-%	70	%	ChE	2911	IU/l
PT-INR	1.23		T-cho	225	mg/dl
APTT	27	s	TG	122	mg/dl
Fib	436		T-bil	0.5	mg/dl
HPT	78	%	D-bil	0.2	mg/dl
AT-III	95	%	TP	7.1	g/dl
Na	137	mEq/l	Alb	3.8	g/dl
K	4.3	mEq/l	AFP	2064	ng/ml
Cl	102	mEq/l	PIVKA-II	374136	mAU/ml
UN	13	mg/dl	HBsAg	(+)	
Cre	1	mg/dl	HBsAb	(−)	
			HBeAg	(+)	
			HBeAb	(+)	
			HCV-Ab	(−)	

Only serum AFP and PIVKA-II levels were high

WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; Ht, hematocrit; Plt, platelet count; PT, prothrombin time; INR, international normalized ratio; APTT activated partial thromboplastin time; Fib, fibrinogen; HPT, hepaplastin test; AT-III, antithrombin III; UN, urea nitrogen; Cre, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ChE, choline esterase; T-cho, total cholesterol; TG, triglycerides; T-bil, total bilirubin; D-bil, direct bilirubin; TP, total protein; Alb, albumin; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist II; HBsAg, hepatitis B surface antigen; HBsAb, antibody to hepatitis B surface antigen; HBeAg; hepatitis B e antigen; HBeAb, antibody to hepatitis B e antigen; HCV-Ab; antibody to hepatitis C virus

with PVTT with a radical operation followed by combined chemotherapy of IFN-α and 5-FU.[5] The patient underwent extended left and caudate lobectomy of the liver and extirpation of the PVTT in July 2003. Forty days after the operation, combined chemotherapy of a subcutaneous injection of 5×10^6 units of IFN-α and an intra-arterial infusion of 300 mg/mm² per day of 5-FU was started. IFN-α was administered 3 days a week for 4 weeks, and 5-FU was infused continuously for 2 weeks using a subcutaneous injection port that carried to the proper hepatic artery, as we previously reported.[6-8] After two cycles of combination chemotherapy with IFN-α and 5-FU, abdominal computed tomography (CT) revealed growth of a recurrent lesion in the remnant liver, and a transcatheter chemoembolization (TAE) was performed in October 2003. Seven months after the operation, the level of protein induced by vitamin K absence or antagonist (PIVKA)-II increased to 47 080 mAU/ml, and multiple remnant liver recurrences, metastasis in the spleen 7 cm in size, and pulmonary metastasis in

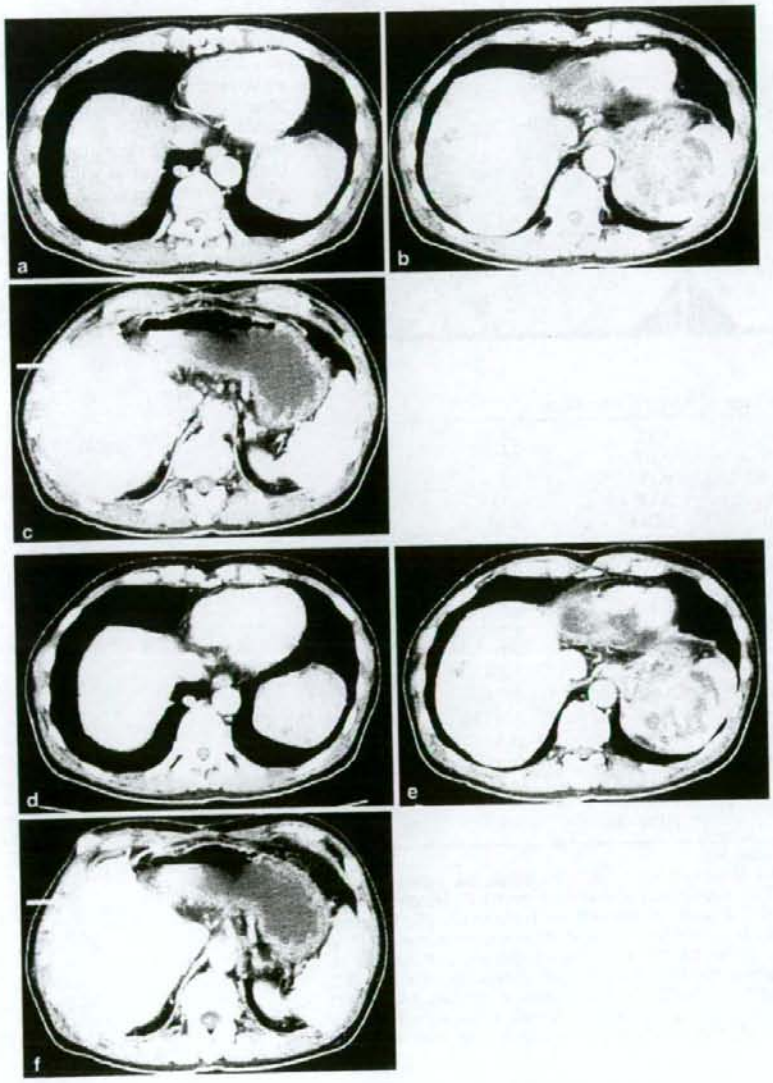

Fig. 2a–f. Abdominal computed tomography in early phase (**a–c**) and late phase (**d–f**) performed before S-1/interferon (IFN)-α administration. **a** Pulmonary metastasis was found in segment 10 of the right lung (*arrow*). A large volume of metastasis was seen in the spleen. **b** The size of splenic metastasis was 7 cm. **c** Multiple remnant liver recurrences were detected (*arrows*). **d–f** The enhancements were washed out from the metastatic lesions (*arrows*)

segment 10 of the right lung were detected on CT (Fig. 2). We started combined chemotherapy of S-1 and IFN-α as a systemic therapy. S-1 was orally administered, 80 mg/day daily, and 5×10^6 units of IFN-α was injected subcutaneously 3 times a week for 4 weeks as one cycle, in our outpatient clinic. After two cycles, CT revealed excellent efficacy of this treatment: lesions in the remnant liver and spleen had disappeared, which was diagnosed as complete remission, in radiological examination; and pulmonary metastasis showed partial remission (Fig. 3). The serum levels of the tumor markers decreased after administration of S-1/IFN-α (Fig. 4). No adverse effect was seen during this treatment. Following the combined chemotherapy with S-1 and IFN-α, the patient underwent partial resection of the right lung for a solitary metastasis in segment 1, 28 months after the initial operation. This patient has survived 32 months since the first operation and is now able to work full-time with no sign of relapse in terms of tumor markers or imaging modalities.

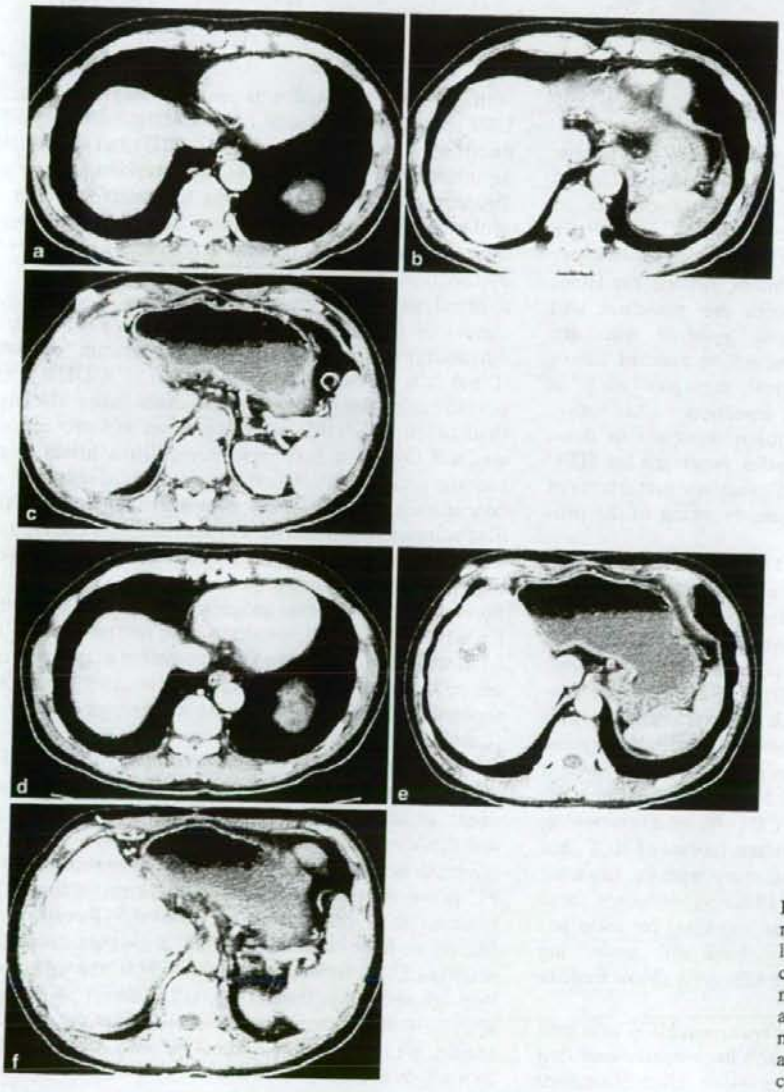

Fig. 3a–f. Abdominal computed tomography in early phase (**a–c**) and late phase (**d–f**) performed after two courses of S-1/IFN-α. **a, d** Pulmonary metastasis decreased in size and a partial response was diagnosed. **b, c, e, f** Tumors in the spleen and remnant liver had disappeared completely

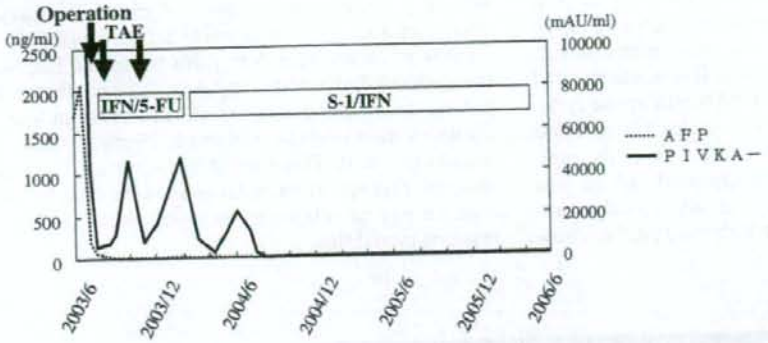

Fig. 4. Serum levels of α-fetoprotein (*AFP*) and protein induced by vitamin K absence or antagonist (*PIVKA-II*) during the treatment. The level of PIVKA-II decreased after the administration of S-1/IFN. *TAE*, transcatheter chemoembolization; *5-FU*, 5-fluorouracil

Discussion

HCC is a common malignancy worldwide and is now the third major cause of death in Japan.[13] In the United States, among approximately 3 million carriers of HCV, cirrhosis will develop in 20%–30%, and among those, HCC will likely develop at a rate of 3%–5% per year.[14] There are several treatment options for HCC, but the only curative therapies are resection and liver transplantation. In those patients who are not candidates for these therapies because of tumor spread or liver function, local therapies such as chemoembolization, alcohol injection, and radiofrequency ablation can be chosen. Progress in these local therapies has led to a better prognosis for HCC patients. On the other hand, extrahepatic metastases of HCC are observed more frequently owing to the prolonged survival of patients.

Extrahepatic metastases of HCC are reported to occur in 13.5%–37% of patients with HCC, and the prognosis of these patients is extremely poor.[15,16] The lack of an effective therapy may be one reason for these insufficient results. Pulmonary metastasis is most frequent, accounting for more than 50% of extrahepatic metastases, but in only 3% of such patients is surgery indicated.[16] To treat metastatic lesions in multiple organs, only systemic chemotherapy might be effective. Several chemotherapeutic drugs, 5-FU, doxorubicin, cis-diamminedichloroplatinum (CDDP), and etoposide, have been used for such metastatic lesions of HCC, but the results have not been satisfactory, with the reported response rates not exceeding 10%.[17–21] Moreover these cytotoxic anticancer agents are too toxic for most patients with HCC, particularly those with underlying chronic liver dysfunction. High efficacy and low toxicity are needed in such cases.[22]

We previously reported one recurrent HCC case with uncontrollable multiple lung and bone metastases that showed almost complete regression after treatment with UFT and IFN-α; this patient survived for 7 years.[23] UFT is the combination of 1 M 1-(2-tetrahydrofuryl)-5-fluorouracil (tegafur, a prodrug of 5-FU) and 4 M uracil, an inhibitor of DPD.[24] DPD is a metabolic enzyme of fluorouracil, and a DPD inhibitor is expected result in a prolonged high serum and tissue 5-FU concentration, thus enhancing the antitumor effect of 5-FU by RNA dysfunction and damage to DNA synthesis. S-1 is a novel per oral combination anticancer drug that consist of tegafur and two modulators, 5-chloro-2, 4 dihydroxypyridine (CDHP) and potassium oxonate (Oxo) at a molecular ratio of 1 : 0.4 : 1.[25] CDHP is reported to be approximately 200 times more effective than uracil as a DPD inhibitor, from in vitro experiments.[26] Oxo is a reversible competitive inhibitor of orotate phosphoribosyltransferase that is reported to concentrate selectively in gastrointestinal tissues after oral administration and to suppress gastrointestinal toxicity caused by phosphoribosylation of 5-FU in the gastrointestinal tract without decreasing antitumor activity. Moreover, S-1 is an oral anticancer agent, and it has the big advantage that hospitalization is not needed for its administration. S-1 is used for treatment of gastric cancer in Japan, and excellent antitumor activity has been reported in phase II studies as a single agent and in combination therapy.[27,28]

In this case, we used combination chemotherapy of S-1 and IFN-α. Even though S-1 is an effective anticancer agent, its use is not promising against HCC as a single agent, because the antitumor component of S-1, tegafur, is a prodrug of 5-FU. Several studies have shown that 5-FU is not effective against HCC in terms of tumor regression and prolongation of survival.[3,4] Recently, we and others have clarified that 5-FU shows antineoplastic activity only in combination with IFN-α, synergistically through several pathways, including direct cell arrest, apoptosis induction, and immunomodulation. For this reason, we used S-1 in combination with IFN-α and not as a single agent.[5–12]

In the clinical response, S-1/IFN-α showed excellent efficacy against the distant metastatic tumors. Thus, S-1 successfully maintained the concentration of fluorouracil in major circulation and showed antitumor activity in its interaction with IFN-α. In addition, lesions in the remnant liver also showed complete remission, even though the combination of an intra-arterial infusion of 5-FU and IFN-α had no effect. This fact suggests that CDHP effectively inhibited DPD. This DPD inhibitory effect might have played a role in the effect on distant metastatic lesions.

In conclusion, we report a case of HCC with multiple lung and spleen metastasis, successfully treated by combination therapy with the novel DPD inhibitory per oral anticancer agent S-1 and IFN-α. The combination therapy of TS-1 and IFNα may be a promising treatment modality against advanced HCC with distant metastasis.

References

1. Farmer DG, Rosove MH, Shaked A, Busuttil RW. Current treatment modalities for hepatocellular carcinoma. Ann Surg 1994;219:236–47.
2. Ikai I, Yamaoka Y, Yamamoto Y, Ozaki N, Sakai Y, Satoh S, et al. Surgical intervention for patients with stage IV-A hepatocellular carcinoma without lymph node metastasis: proposal as a standard therapy. Ann Surg 1998; 227:433–9.
3. Shen DW, Lu YG, Chin KV, Pastan I, Gottesman MM. Human hepatocellular carcinoma cell lines exhibit multidrug resistance unrelated to MRD1 gene expression. J Cell Sci 1991;98:317–22.
4. Doci R, Bignami P, Bozzetti F, Bonfanti G, Audisio R, Colombo M, et al. Intrahepatic chemotherapy for unresectable hepatocellular carcinoma. Cancer 1988;61:1983–7.
5. Urabe T, Kaneko S, Matsushita E, Unoura M, Kobayashi K. Clinical pilot study of intrahepatic arterial chemotherapy with methotrexate, 5-fluorouracil, cisplatin and subcutaneous interferon-α-2b for patients with locally advanced hepatocellular carcinoma. Oncology 1998;55:39–47.
6. Sakon M, Nagano H, Dono K, Nakamori S, Umeshita K, Yamada A, et al. Combined intraarterial 5-fluorouracil and subcutaneous interferon-α therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. Cancer 2002;94:435–42.
7. Ota H, Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, et al. Treatment of hepatocellular carcinoma with major portal vein thrombosis by combination of subcutaneous interferon-α and intra-arterial 5-fluorouracil. Role of expression of type 1 interferon receptor. Br J Cancer 2005;93:557–64.
8. Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, Ota H, et al. Hepatic resection followed by IFN-α and 5-FU for advanced hepatocellular carcinoma with tumor thrombus in the major portal branch. Hepatogastroenterology (in press).
9. Obi S, Yoshida H, Toune R, Unuma T, Kanda M, Sato S, et al. Combination therapy of intra-arterial 5-fluorouracil and systemic interferon-α for advanced hepatocellular carcinoma with portal venous invasion. Cancer 2006;106:1990–7.
10. Eguchi H, Nagano H, Yamamoto H, Miyamoto A, Kondo M, Dono K, et al. Augmentation of antitumor activity of 5-fluorouracil by interferon α is associated with up-regulation of p27Kip1 in human hepatocellular carcinoma cells. Clin Cancer Res 2000;6:2881–90.
11. Yamamoto T, Nagano H, Sakon M, Wada H, Eguchi H, Kondo M, et al. Partial contribution of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/TRAIL receptor pathway to antitumor effects of interferon-α/5-fluorouracil against hepatocellular carcinoma. Clin Cancer Res 2004;10:7884–95.
12. Kondo M, Nagano H, Wada H, Damdinsuren B, Yamamoto H, Hiraoka N, et al. Combination of IFN-α and 5-fluorouracil induces apoptosis through IFN-α/β receptor in human hepatocellular carcinoma cells. Clin Cancer Res 2005;11:1277–86.
13. Arii S, Monden K, Niwano M, Furutani M, Mori A, Mizumoto M, et al. Results of surgical treatment for recurrent hepatocellular carcinoma; comparison of outcome among patients with multicentric carcinogenesis, intrahepatic metastasis, and extrahepatic recurrence. J Hepatobiliary Pancreat Surg 1998;5:86–92.
14. Carr BI. Hepatocellular carcinoma: current management and future trends. Gastroenterology 2004;127:S218–24.
15. Katyal S, Oliver JH III, Peterson MS, Ferris JV, Carr BS, Baron RL. Extrahepatic metastases of hepatocellular carcinoma. Radiology 2000;216:698–703.
16. Natsuizaka M, Omura T, Akaike T, Kuwata Y, Yamazaki K, Sato T, et al. Clinical features of hepatocellular carcinoma with extrahepatic metastases. J Gastroenterol Hepatol 2005;20:1781–7.
17. Friedman MA. Primary hepatocellular cancer-present results and future prospects. Int J Radiat Oncol Biol Phys 1983;9:1841–50.
18. Lin DY, Lin SM, Liaw YF. Non-surgical treatment of hepatocellular carcinoma. J Gastroenterol Hepatol 1997;12:S319–28.
19. Lai CL, Wu PC, Chan CG, Lok AS, Lin HJ. Doxorubicin versus no antitumor therapy in inoperable hepatocellular carcinoma. A prospective randomized trial. Cancer 1988;62:479–83.
20. Nerenstone SR, Ihde DC, Friedman MA. Clinical trials in primary hepatocellular carcinoma: current status and future directions. Cancer Treat Rep 1988;15:1–31.
21. Simonetti RG, Liberati A, Angiolini C, Pagliaro L. Treatment of hepatocellular carcinoma: a systematic review of randomized controlled trials. Ann Oncol 1997;8:117–36.
22. Hoofnagle JH. Hepatocellular carcinoma: summary and recommendations. Gastroenterology 2004;127:S319–23.
23. Miyamoto A, Umeshita K, Sakon M, Nagano H, Eguchi H, Kishimoto S, et al. Advanced hepatocellular carcinoma with distant metastasis, successfully treated by combination therapy of α-interferon and oral tegafur/uracil. J Gastroenterol Hepatol 2000;15:1447–51.
24. Taguchi T. Clinical application of biochemical modulation in cancer chemotherapy: biological modulation for 5-FU. Oncology 1997;54:19–23.
25. Shirasaka T, Nakano K, Takechi T, Satake H, Uchida J, Fujioka A, et al. Antitumor activity of 1 M tegafur–0.4 M 5-chloro-2,4-dihydroxypyridine–1 M potassium oxanate (S-1) against human colon carcinoma orthotopically implanted into nude rats. Cancer Res 1996;56:2602–6.
26. Tatsumi K, Fukushima M, Shirasaka T, Fujii S. Inhibitory effects of pyrimidine, barbituric acid and pyrimidine derivatives on 5-fluorouracil degeneration in rat liver extracts. Jpn J Cancer Res 1987;78:748–55.
27. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T. Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur–0.4 M gimestat–1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 1998;34:1715–20.
28. Nakata B, Mitachi Y, Tsuji A, Yamamitsu S, Hirata K, Shirasaka T, et al. Combination phase I trial of novel oral fluorouracil derivative S-1 with low-dose cisplatin for unresectable and recurrent gastric cancer (JFMC27–9902). Clin Cancer Res 2004;10:1664–9.

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Clinical Studies

Expression pattern of angiogenic factors and prognosis after hepatic resection in hepatocellular carcinoma: importance of angiopoietin-2 and hypoxia-induced factor-1α

Wada H, Nagano H, Yamamoto H, Yang Y, Kondo M, Ota H, Nakamura M, Yoshioka S, Kato H, Damdinsuren B, Tang D, Marubashi S, Miyamoto A, Takeda Y, Umeshita K, Nakamori S, Sakon M, Dono K, Wakasa K and Monden M. Expression pattern of angiogenic factors and prognosis after hepatic resection in hepatocellular carcinoma: importance of angiopoietin-2 and hypoxia-induced factor-1α.
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Abstract: *Background:* Hepatocellular carcinoma (HCC) is a hypervascular tumor and angiogenesis plays an important role in its progression. Angiogenesis is regulated by a balance between pro and antiangiogenic molecules. The aim of this study was to investigate the expressions of angiogenic factors and elucidate their roles in angiogenesis in HCC. *Methods:* We investigated immunohistochemical expression of vascular endothelial growth factor (VEGF), angiopoietins (Ang-1 and Ang-2), hypoxia-induced factor-1α (HIF-1α) and thrombospondin-1 (TSP-1) in 60 specimens of surgically resected HCC. We investigated the relationship between their expressions and clinicopathological factors or prognosis. *Results:* Ang-2 staining had a significant correlation with the grade of differentiation of HCC cells ($P = 0.0082$). VEGF and Ang-2 expression correlated positively with microvessel density (MVD) ($P = 0.0061$ and 0.0374, respectively). MVD of well-differentiated HCC were significantly lower than those of moderately and poorly differentiated HCC. The disease-free survival time of patients with high Ang-2 and/or HIF-1α expression was significantly shorter than that of the low expression group ($P = 0.0278$ and 0.0374, respectively). *Conclusion:* Our study showed that the expression of VEGF and Ang-2 correlated with MVD. Strong Ang-2 expression and/or high nuclear expression of HIF-1α is a significant predictive factor for recurrence after curative resection in HCC patients.

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Key words: angiogenesis – angiopoietin – hepatocellular carcinoma – hypoxia-induced factor – vascular endothelial growth factor

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Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world, especially in Japan and other East Asian countries. HCC is characteristically a highly vascular tumor with a propensity for vascular invasion and intrahepatic metastasis. Hepatic resection is considered a curative treatment for HCC, but the prognosis after resection remains unsatisfactory because of the high incidence of recurrence (1). In various cancers, including HCC, tumor angiogen-

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Abbreviation: Ang, angiopoietin; ARNT, aryl hydrocarbon receptor nuclear translocator; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HIF, hypoxia-induced factor; MVD, microvessel density; PBS, phosphate-buffered saline; TSP-1, thrombospondin-1; VEGF, vascular endothelial growth factor.

In the clinical response, S-1/IFN-α showed excellent efficacy against the distant metastatic tumors. Thus, S-1 successfully maintained the concentration of fluorouracil in major circulation and showed antitumor activity in its interaction with IFN-α. In addition, lesions in the remnant liver also showed complete remission, even though the combination of an intra-arterial infusion of 5-FU and IFN-α had no effect. This fact suggests that CDHP effectively inhibited DPD. This DPD inhibitory effect might have played a role in the effect on distant metastatic lesions.

In conclusion, we report a case of HCC with multiple lung and spleen metastasis, successfully treated by combination therapy with the novel DPD inhibitory per oral anticancer agent S-1 and IFN-α. The combination therapy of TS-1 and IFNα may be a promising treatment modality against advanced HCC with distant metastasis.

References

1. Farmer DG, Rosove MH, Shaked A, Busuttil RW. Current treatment modalities for hepatocellular carcinoma. Ann Surg 1994;219:236–47.
2. Ikai I, Yamaoka Y, Yamamoto Y, Ozaki N, Sakai Y, Satoh S, et al. Surgical intervention for patients with stage IV-A hepatocellular carcinoma without lymph node metastasis: proposal as a standard therapy. Ann Surg 1998; 227:433–9.
3. Shen DW, Lu YG, Chin KV, Pastan I, Gottesman MM. Human hepatocellular carcinoma cell lines exhibit multidrug resistance unrelated to MRD1 gene expression. J Cell Sci 1991;98:317–22.
4. Doci R, Bignami P, Bozzetti F, Bonfanti G, Audisio R, Colombo M, et al. Intrahepatic chemotherapy for unresectable hepatocellular carcinoma. Cancer 1988;61:1983–7.
5. Urabe T, Kaneko S, Matsushita E, Unoura M, Kobayashi K. Clinical pilot study of intrahepatic arterial chemotherapy with methotrexate, 5-fluorouracil, cisplatin and subcutaneous interferon-α-2b for patients with locally advanced hepatocellular carcinoma. Oncology 1998;55:39–47.
6. Sakon M, Nagano H, Dono K, Nakamori S, Umeshita K, Yamada A, et al. Combined intraarterial 5-fluorouracil and subcutaneous interferon-α therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. Cancer 2002;94:435–42.
7. Ota H, Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, et al. Treatment of hepatocellular carcinoma with major portal vein thrombosis by combination of subcutaneous interferon-α and intra-arterial 5-fluorouracil. Role of expression of type 1 interferon receptor. Br J Cancer 2005;93:557–64.
8. Nagano H, Sakon M, Eguchi H, Kondo M, Yamamoto T, Ota H, et al. Hepatic resection followed by IFN-α and 5-FU for advanced hepatocellular carcinoma with tumor thrombus in the major portal branch. Hepatogastroenterology (in press).
9. Obi S, Yoshida H, Toune R, Unuma T, Kanda M, Sato S, et al. Combination therapy of intra-arterial 5-fluorouracil and systemic interferon-α for advanced hepatocellular carcinoma with portal venous invasion. Cancer 2006;106:1990–7.
10. Eguchi H, Nagano H, Yamamoto H, Miyamoto A, Kondo M, Dono K, et al. Augmentation of antitumor activity of 5-fluorouracil by interferon α is associated with up-regulation of p27Kip1 in human hepatocellular carcinoma cells. Clin Cancer Res 2000;6:2881–90.
11. Yamamoto T, Nagano H, Sakon M, Wada H, Eguchi H, Kondo M, et al. Partial contribution of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/TRAIL receptor pathway to antitumor effects of interferon-α/5-fluorouracil against hepatocellular carcinoma. Clin Cancer Res 2004;10:7884–95.
12. Kondo M, Nagano H, Wada H, Damdinsuren B, Yamamoto H, Hiraoka N, et al. Combination of IFN-α and 5-fluorouracil induces apoptosis through IFN-α/β receptor in human hepatocellular carcinoma cells. Clin Cancer Res 2005;11:1277–86.
13. Arii S, Monden K, Niwano M, Furutani M, Mori A, Mizumoto M, et al. Results of surgical treatment for recurrent hepatocellular carcinoma; comparison of outcome among patients with multicentric carcinogenesis, intrahepatic metastasis, and extrahepatic recurrence. J Hepatobiliary Pancreat Surg 1998;5:86–92.
14. Carr BI. Hepatocellular carcinoma: current management and future trends. Gastroenterology 2004;127:S218–24.
15. Katyal S, Oliver JH III, Peterson MS, Ferris JV, Carr BS, Baron RL. Extrahepatic metastases of hepatocellular carcinoma. Radiology 2000;216:698–703.
16. Natsuizaka M, Omura T, Akaike T, Kuwata Y, Yamazaki K, Sato T, et al. Clinical features of hepatocellular carcinoma with extrahepatic metastases. J Gastroenterol Hepatol 2005;20:1781–7.
17. Friedman MA. Primary hepatocellular cancer-present results and future prospects. Int J Radiat Oncol Biol Phys 1983;9:1841–50.
18. Lin DY, Lin SM, Liaw YF. Non-surgical treatment of hepatocellular carcinoma. J Gastroenterol Hepatol 1997;12:S319–28.
19. Lai CL, Wu PC, Chan CG, Lok AS, Lin HJ. Doxorubicin versus no antitumor therapy in inoperable hepatocellular carcinoma. A prospective randomized trial. Cancer 1988;62:479–83.
20. Nerenstone SR, Ihde DC, Friedman MA. Clinical trials in primary hepatocellular carcinoma: current status and future directions. Cancer Treat Rep 1988;15:1–31.
21. Simonetti RG, Liberati A, Angiolini C, Pagliaro L. Treatment of hepatocellular carcinoma: a systematic review of randomized controlled trials. Ann Oncol 1997;8:117–36.
22. Hoofnagle JH. Hepatocellular carcinoma: summary and recommendations. Gastroenterology 2004;127:S319–23.
23. Miyamoto A, Umeshita K, Sakon M, Nagano H, Eguchi H, Kishimoto S, et al. Advanced hepatocellular carcinoma with distant metastasis, successfully treated by combination therapy of α-interferon and oral tegafur/uracil. J Gastroenterol Hepatol 2000;15:1447–51.
24. Taguchi T. Clinical application of biochemical modulation in cancer chemotherapy: biological modulation for 5-FU. Oncology 1997;54:19–23.
25. Shirasaka T, Nakano K, Takechi T, Satake H, Uchida J, Fujioka A, et al. Antitumor activity of 1 M tegafur–0.4 M 5-chloro-2,4-dihydroxypyridine–1 M potassium oxanate (S-1) against human colon carcinoma orthotopically implanted into nude rats. Cancer Res 1996;56:2602–6.
26. Tatsumi K, Fukushima M, Shirasaka T, Fujii S. Inhibitory effects of pyrimidine, barbituric acid and pyrimidine derivatives on 5-fluorouracil degeneration in rat liver extracts. Jpn J Cancer Res 1987;78:748–55.
27. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T. Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur–0.4 M gimestat–1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 1998;34:1715–20.
28. Nakata B, Mitachi Y, Tsuji A, Yamamitsu S, Hirata K, Shirasaka T, et al. Combination phase I trial of novel oral fluorouracil derivative S-1 with low-dose cisplatin for unresectable and recurrent gastric cancer (JFMC27–9902). Clin Cancer Res 2004;10:1664–9.